CYTOKINE PRODUCTION BY CELL CULTURES ON THE INTERNATIONAL SPACE STATION (ISS) USING THE CELLULAR BIOTECHNOLOGY OPERATIONS SUPPORT SYSTEM (CBOSS)

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ABSTRACT

CBOSS is a stationary bioreactor incubator system developed by the Cellular Biotechnology Program for the cultivation of cells aboard the ISS. Of the four cell culture types grown during the ISS Expedition 3 mission, two were found to produce substantial amounts of Interleukins IL-6 and IL-8. This study focuses on the differential production of cytokines by human renal cortical epithelial (HRCE) cells, which were grown in DMEM/F12 culture media with 10% fetal bovine sera in microgravity or on the ground. Initial measurements compared cytokines secreted in the culture media by HRCE cells grown in tissue culture flasks or in the CBOSS Tissue Culture Modules (TCMs). The cells produced increasing amounts of IL-6 and IL-8 in the TCMs and tissue culture flasks during a time course of two days, with less production of both cytokines in the flask vessels. Concentration measurements were done using the Luminex ^{100} bioanalyzer and cytokine kits made by R&D. The spent media from the ISS cell cultures and the matched ground controls were stored refrigerated for 3 months and then frozen until processed. This media contained amounts of IL-6 and IL-8 interleukins similar to freshly obtained, frozen and thawed samples. The samples tested were from media exchanged on day 2, 3, 6 and the culture terminated on day 9. The HRCE cells produced increasing amounts of both IL-6 and IL-8 in both ground and flight cultures. The flight cultures exhibited less cell growth and also less cytokine production. (Supported by NASA Biotechnology Contract NAS9-97114 and NRA grant #NAG8-1362).

INTRODUCTION

During Expedition 3 on the ISS, cell cultures were grown in the CBOSS which is a stationary bioreactor incubator system developed by the Cellular Biotechnology Program at Johnson Space Center. The cell cultures were grown in Tissue Culture Modules (TCM), which are gas permeable Teflon® bags (American Fluoroseal) that discourage cell attachment. Substrate dependent cells are grown on tissue culture beads in the TCMs. Of the four cell cultures grown during Expedition 3 (7A.1), two were found to produce substantial amounts of interleukins IL-6 and IL-8. This report focuses on production of cytokines by the human renal cortical epithelial (HRCE) cells, which were grown in DMEM/F12 media with 10% fetal bovine sera in microgravity or on the ground. Initial study compared cytokines secreted into the culture media by HRCE cells grown in tissue culture flasks or in the CBOSS TCMs on the ground. The cells produced increasing amounts of IL-6 and IL-8 in the TCMs and tissue culture flasks during a time course of two days. During this time there was less production of both cytokines in the tissue culture flasks. The concentration of cytokines was measured using the Luminex ^{100} ® bioanalyzer and cytokine kits made by R&D. Next we examined the spent media from the ISS cell cultures and the matched ground controls which were stored refrigerated for 3 months and then frozen until processed. This media contained amounts of IL-6 and IL-8 cytokines similar to freshly obtained,

frozen and thawed samples. The samples shown here were from media collected on day 2, 3, 6, and when the culture was terminated on day 9. The HRCE cells produced increasing amounts of both IL-6 and IL-8 in both ground and flight cultures over time. The flight cultures exhibited less cell growth than the ground controls as determined by visual inspection of the fixed cells and the lower glucose utilization. The flight samples correspondingly produced lower concentrations of both IL-6 and IL-8.

METHODS

- Materials and equipment used: Cytodex-3 beads (Sigma); Teflon bags (American Fluoroseal); LMAT kit and antibodies for IL-8 and IL-6 (R & D); Luminex ^{100}; DMEM/F12 (Life Technologies) with 10% fetal bovine sera (Hyclone); Ciprofloxacin®, Fungizone®, and $NaHCO_3$ (Life Technologies); and Gentamycin® and HEPES buffer (Sigma).
- Human renal cortical epithelial (HRCE) cells(1) were grown in 15mL media in tissue culture modules (TCMs). Four TCMs were contained in each QTCMA and placed in the Biotechnology Specimen Temperature Controller (BSTC). Both ground and flight cells were fed on days 2, 3, and 6, with the cultures being terminated on day 9. Initial media was used as the 0 time control. Portable Clinical Blood Analyzer (PCBA) data was collected during flight and ground runs using G3+ and 6+ (iSTAT) cartridges and is reported for the same samples as used for cytokine analysis.
- For fixation, as much media as possible (~10 mL) was removed from the TCM with a 10 mL syringe and 9 mL of formalin was injected into the TCM. The sample was stored in a refrigerator at 4^{o}C or on orbit in the Biotechnology Refrigerator (BTR).
- Media removed when the culture was fed and terminated was refrigerated until after the flight and then frozen at –80^{o}C until use.
- Freshly thawed media was used for cytokine measurements. IL-6 and IL-8 were measured simultaneously using an LMAT kit from R&D. Dilutions were made using the diluent from the kit and the plates were read with a 96 well attachment on the Luminex ^{100}.

CBOSS HARDWARE

Figure 1 – A Quad Tissue Culture Module Assembly (QTCMA) containing TCMs with media and HRCE cells during Expedition 3

Figure 2 – The BSTC with the door open showing the QTCMAs stacked inside. The temperature controller held the cultures at 36 ±1°C with a 5% CO_2 atmosphere.

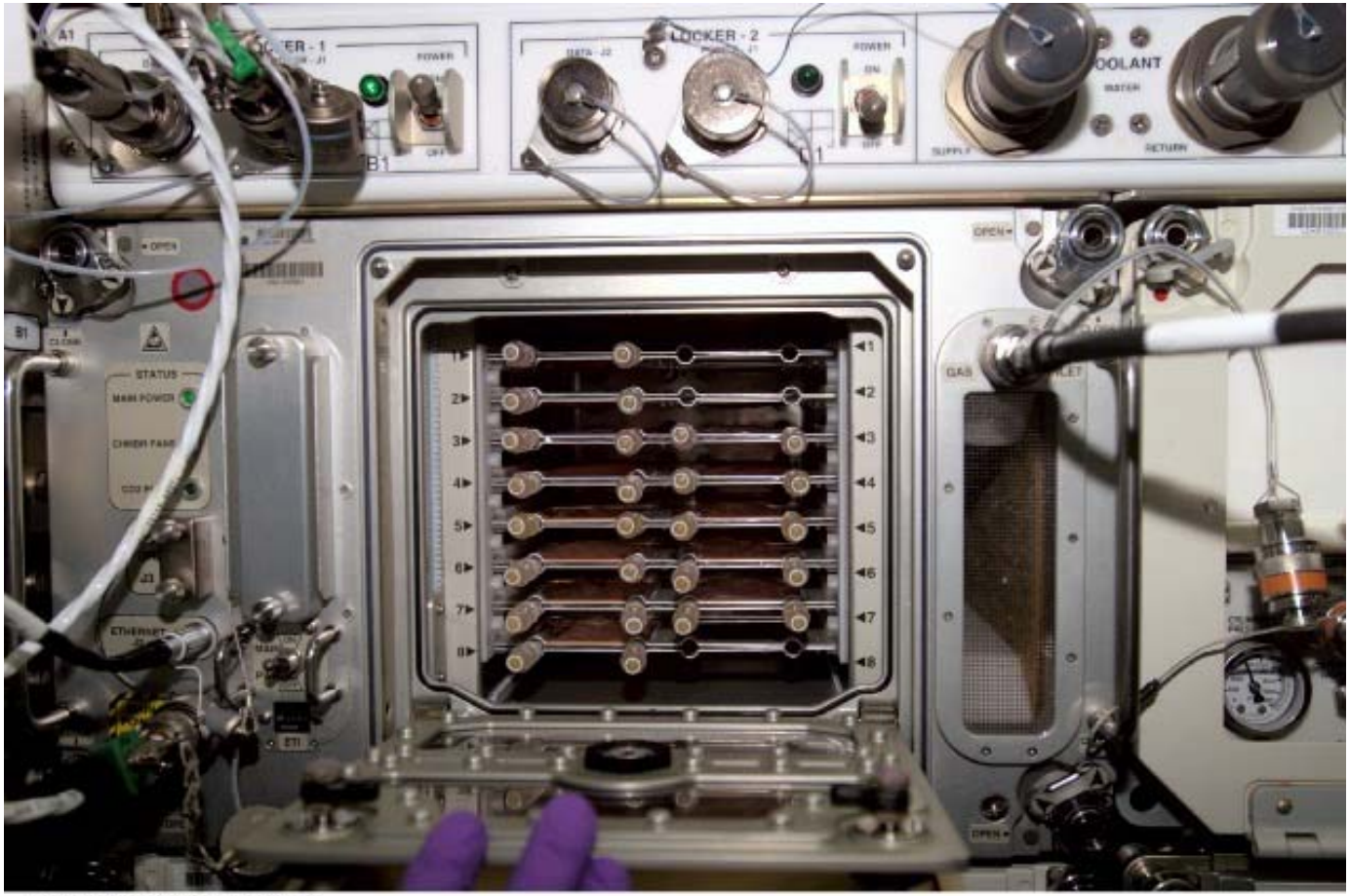


Figure 3 – Astronaut Frank Culbertson examining a QTCMA from the BSTC during Expedition 3

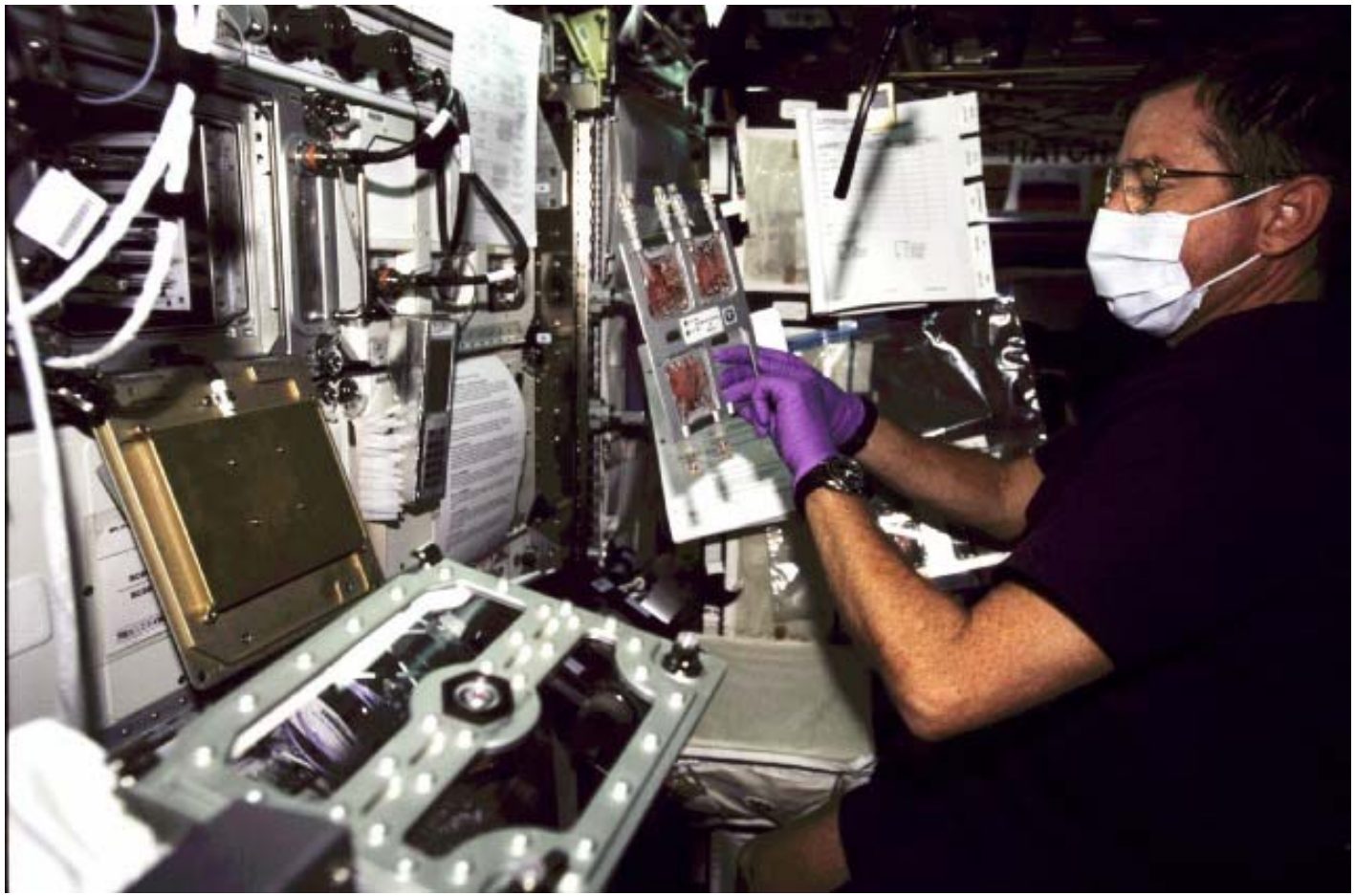
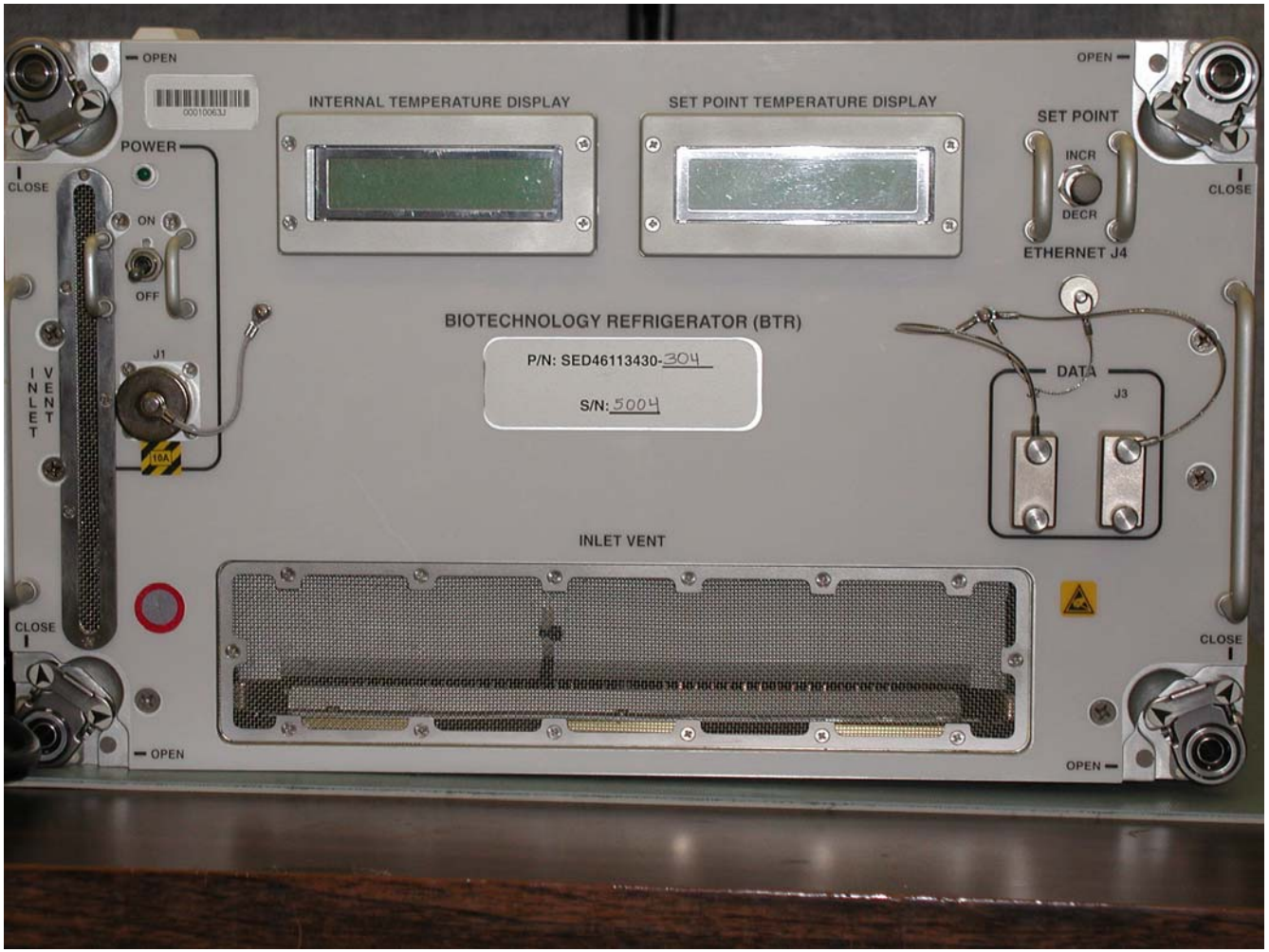


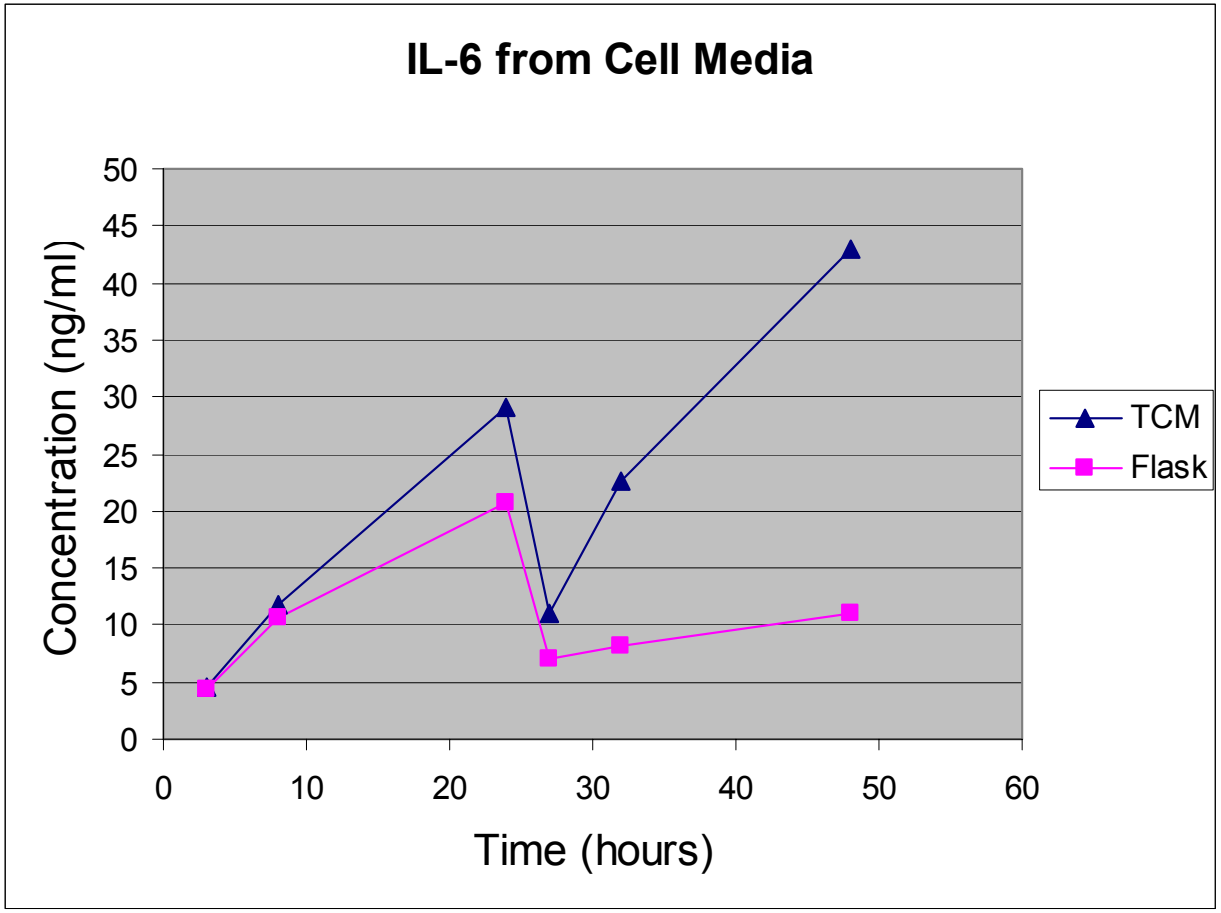
Figure 4 – The Biotechnology Refrigerator (BTR) which is used for refrigerating media and fixed cells at 4^{o}C.



RESULTS AND FIGURES

Figure 5 – Cytokine production by HRCE cells in a ground based experiment demonstrates the increasing content of IL-6 and IL-8 over time. The drop at 28 hours is due to the feeding of the culture at 24 hours. Figure 5A compares the production of IL-6 by cultures in TCMs and tissue culture flasks. Figure 5B compares the production of IL-8 over the same time frame. Both cytokines were produced to a lesser extent in the tissue culture flasks.

5A



5B

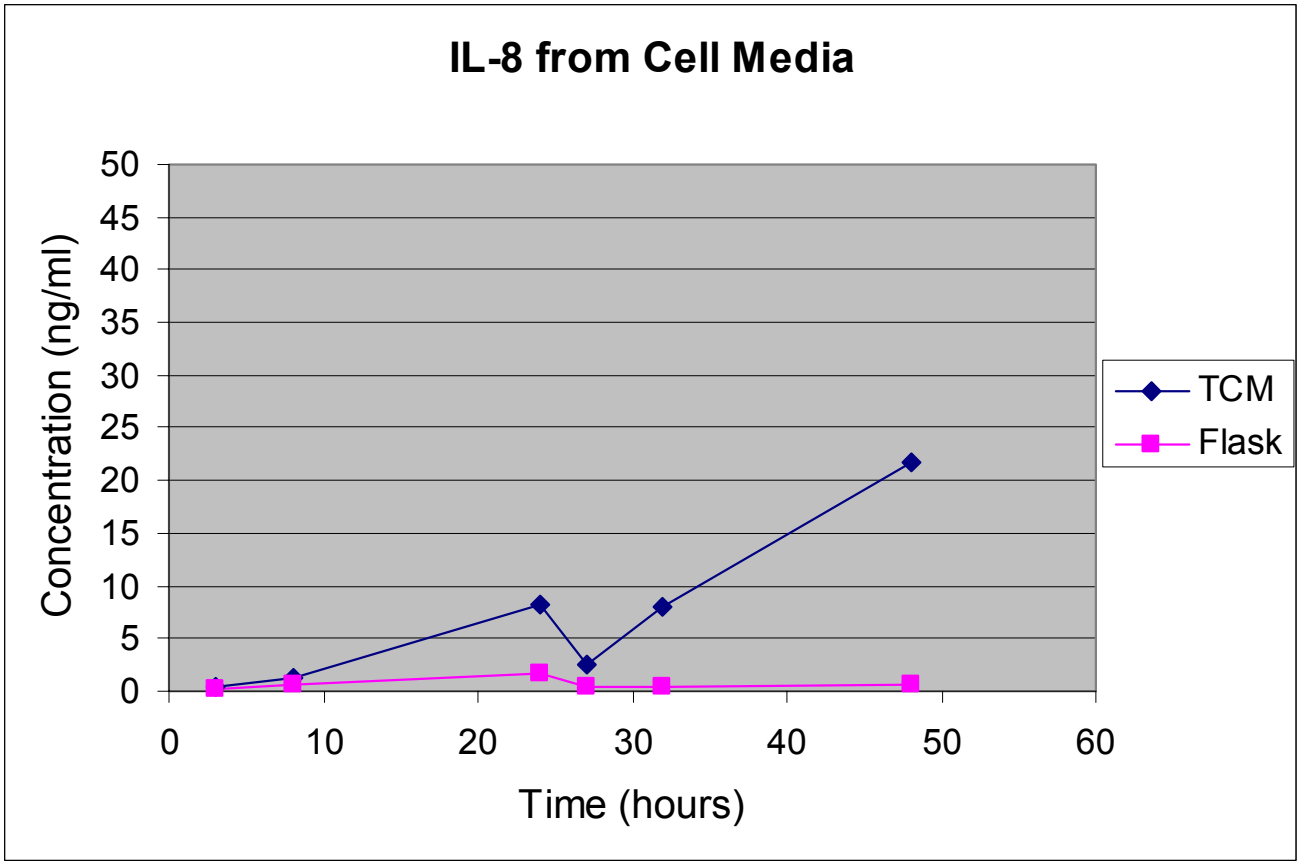

IL-8 from Cell Media
Concentration (ng/ml)
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
Time (hours)
TCM
Flask

Figure 6 – There was an increase in cytokine production in parallel experiments on the ISS (F) and on the ground (G). Both cytokines increased with the IL6 levels remaining slightly higher for all time points. The lower concentrations in the flight experiments reflect the lower growth of cells in the flight experiment.

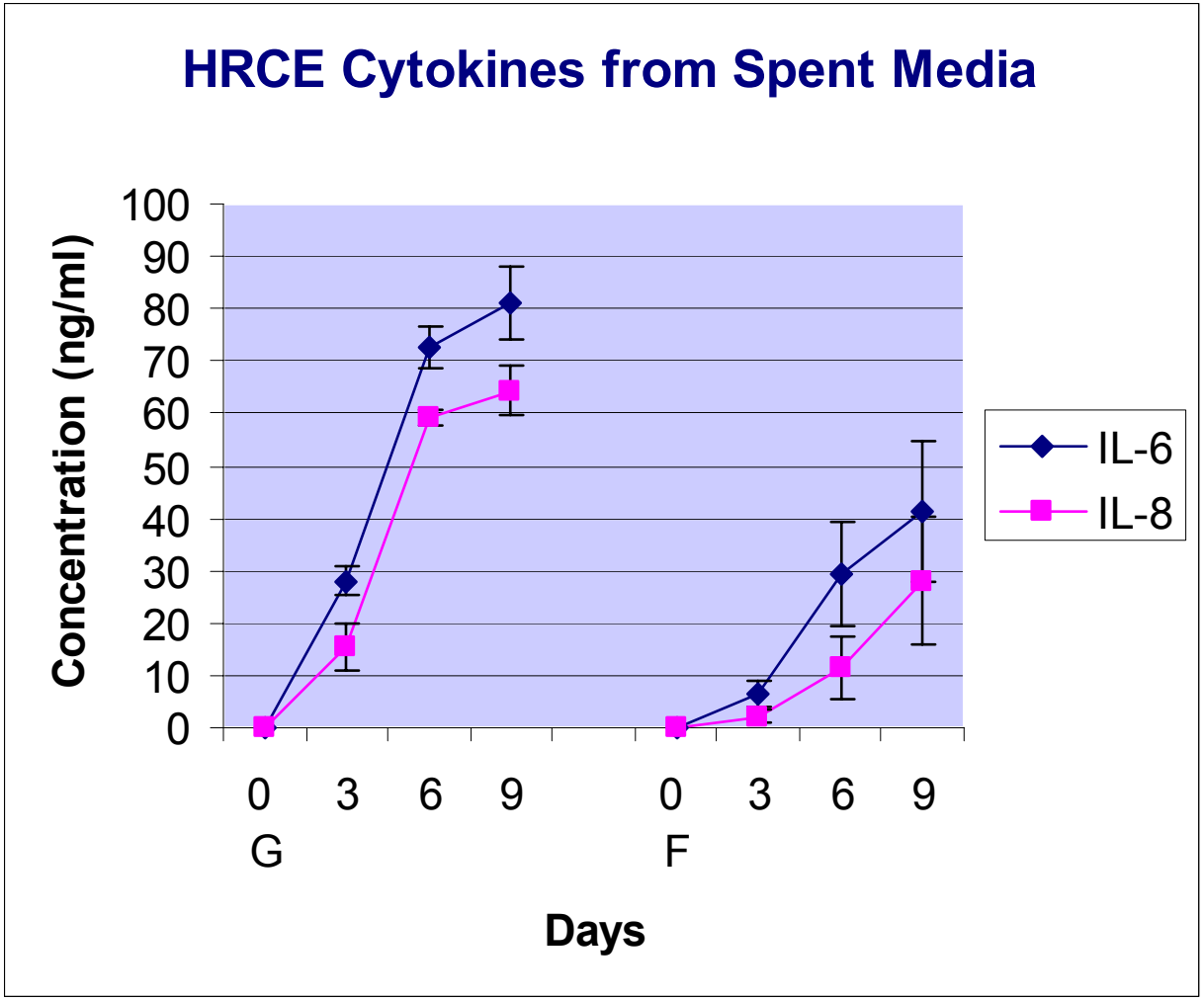


Figure 7 – Picture of the HRCE cells growing on cytodex beads in a TCM from the ground experiment. Note that many of the beads have a ruffled appearance due to cells growing on the beads (100X magnification).

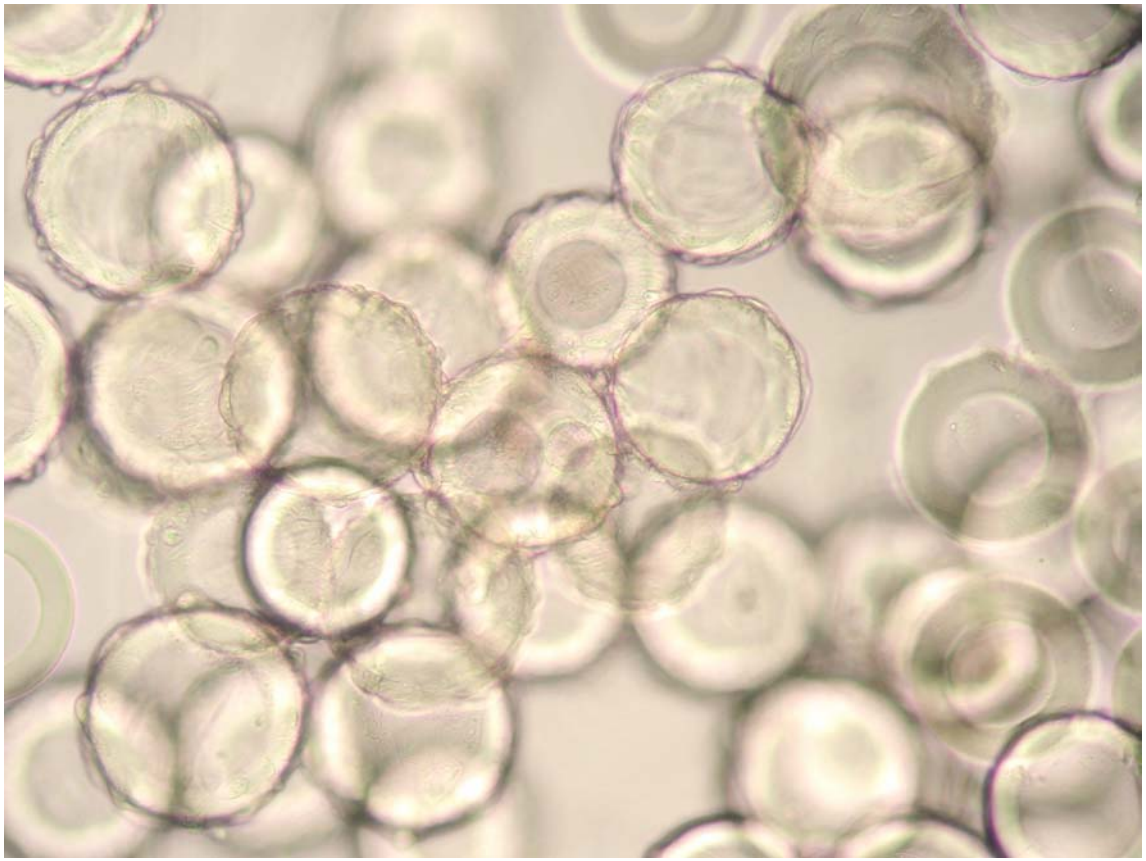


Figure 8 – Picture of the HRCE cells growing on cytodex beads in a TCM from the flight experiment. Note that only a few of the beads have a ruffled appearance. (100X magnification)

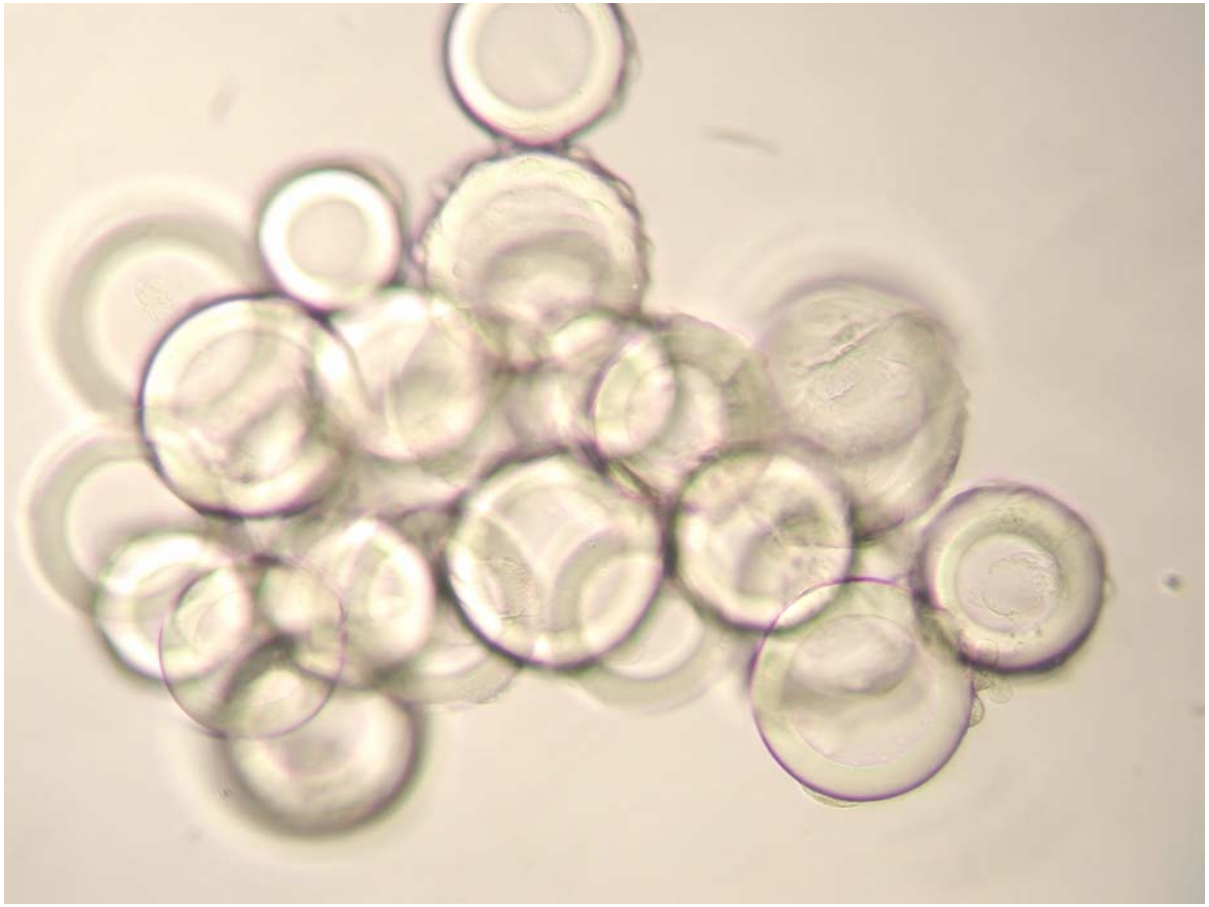


Figure 9 – PCBA data on oxygen and carbon dioxide concentrations. Oxygen and carbon dioxide partial pressures varied over the course of the experiments. Note that the oxygen content of the flight run is increasing beyond the concentration in the ground run, while the carbon dioxide levels are similar.

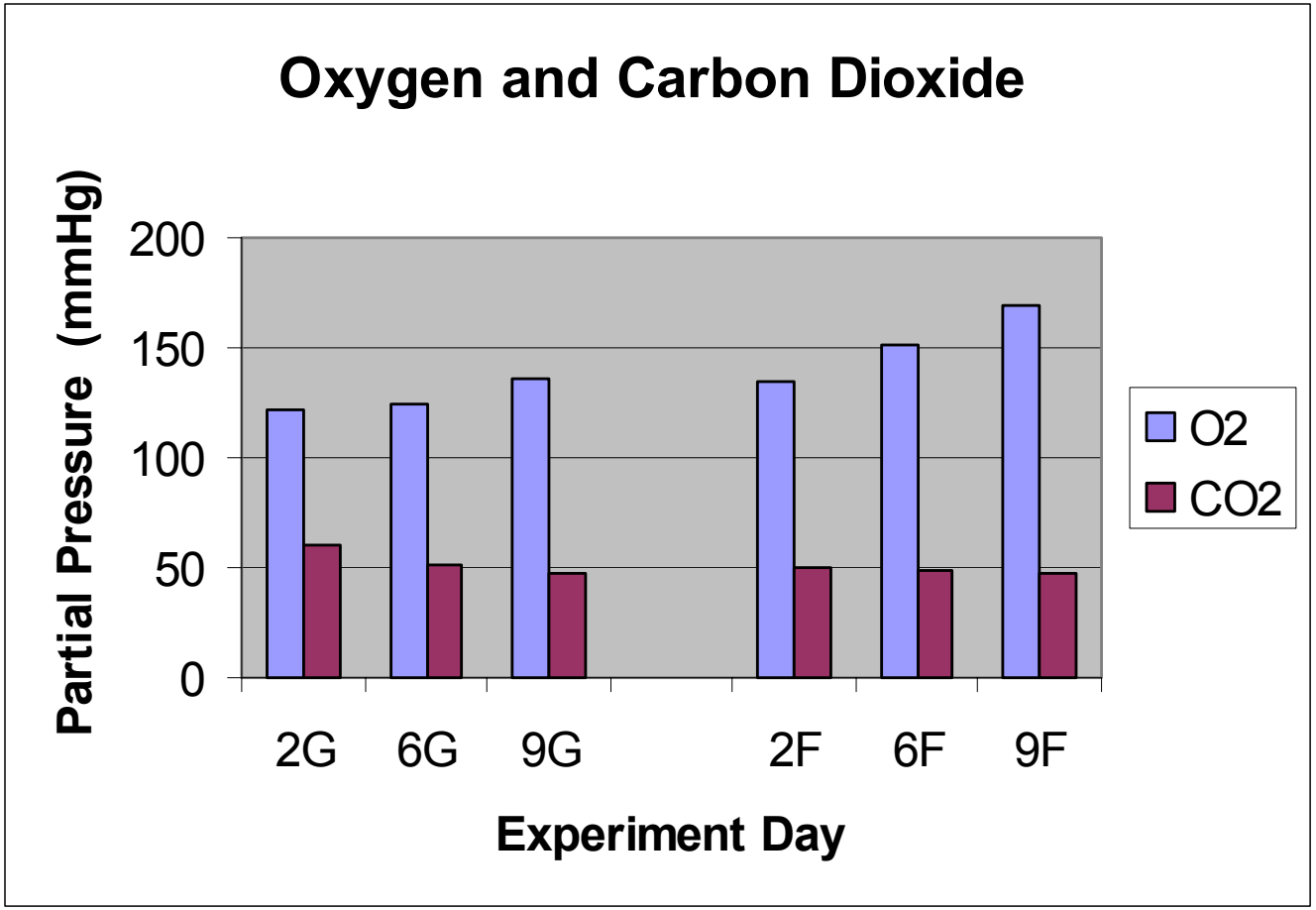


Figure 10 – PCBA data on bicarbonate and pH

Note that although the pH readings are similar, the bicarbonate concentration is higher in the flight samples. The bicarbonate concentration is in mmoles/liter.

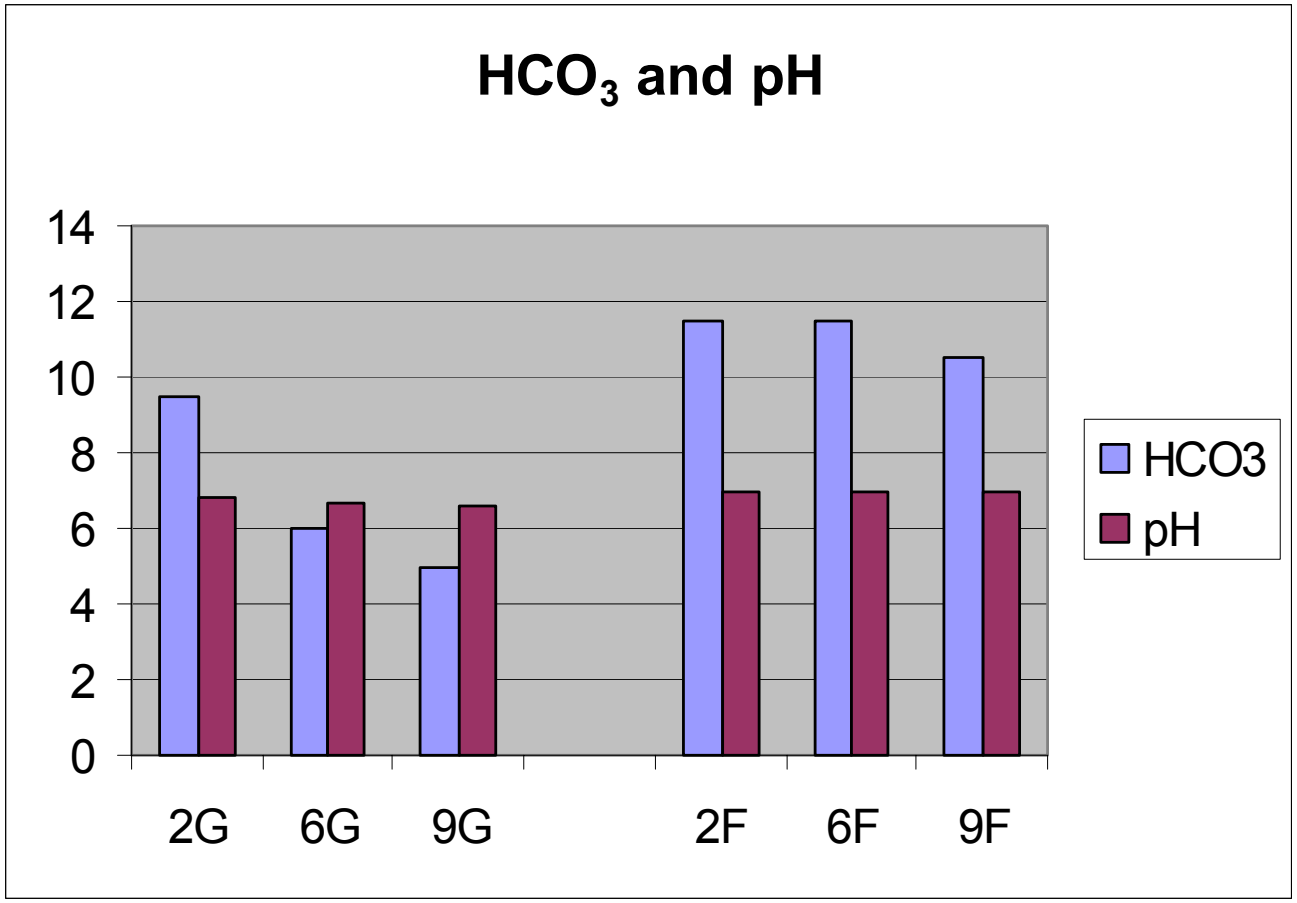
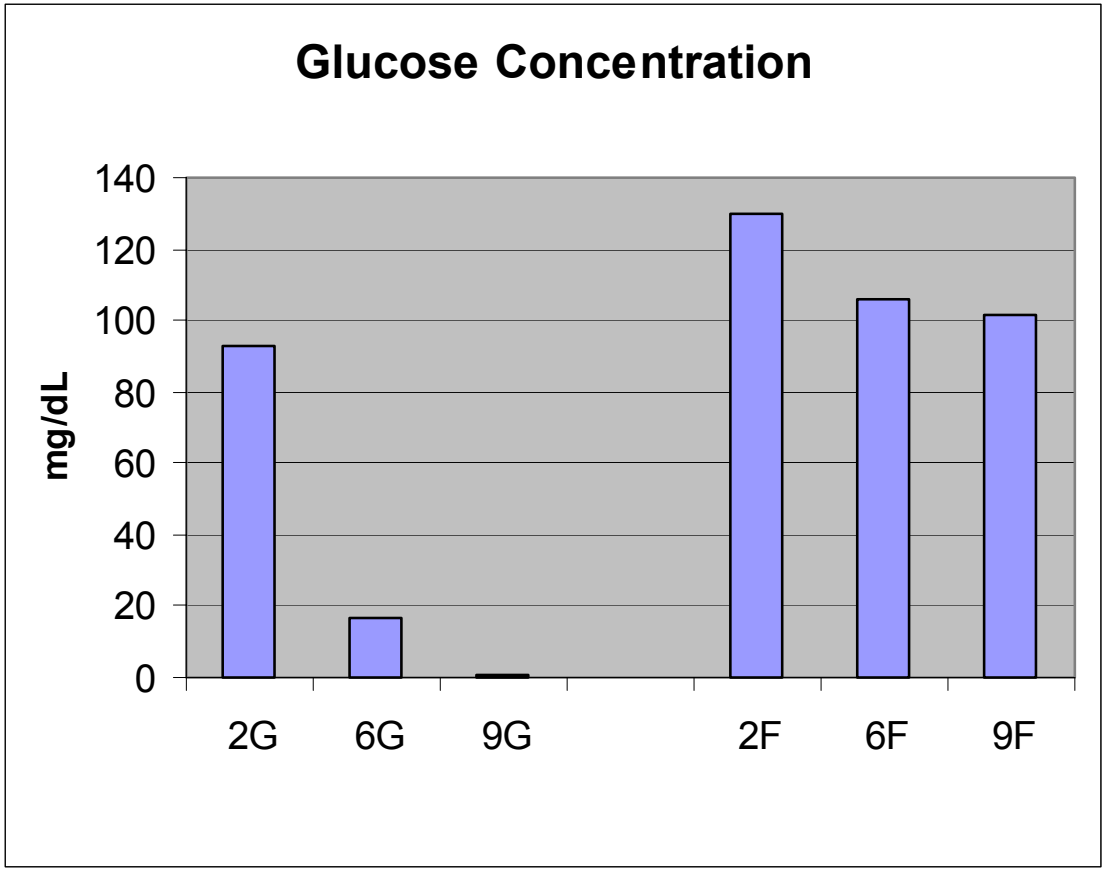


Figure 11 - PCBA data on Glucose

The low glucose consumption again demonstrates that there was less growth in the flight cultures.



CONCLUSIONS

- The production of IL-6 and IL-8 increase over time in culture for the HRCE cells.
- The parallel flight and ground experiments both demonstrated increasing levels of IL-6 and IL-8.
- The flight cells demonstrated a lower level of growth and a lower production of IL-6 and IL-8 than the ground matched control. It is not clear whether this is due to microgravity or to other operational issues.
- The monitoring of cell growth using PCBA data showed differences between the ground and flight runs in the amount of oxygen and bicarbonate in the media.

REFERENCE

1. Cowger,NL, Benes, E, Allen,P and Hammond TG (2002) J Appl Physiol 92:691-700.